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Fungal Communities, Succession, Enzymes, and Decomposition

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I. INTRODUCTION

Fungi are essential for nutrient mobilization, storage, and release during decomposition of plant material in terrestrial ecosystems. Saprophytic microfungi are the least visible group of fungi in soil but are, nevertheless, key decomposers of the massive amounts of leaves, stalks, and other plant parts deposited on and in the ground each year. Because of their hyphal growth pattern, production of vegetative spores, specific survival strategies, and capacity to produce a variety of enzymes important in decomposition processes, these fungi are ubiquitous and respond rapidly to the addition of new substrates.

During the decomposition of plant material the composition of the fungal community changes, a process referred to as *microbial succession* (1). This succession can be viewed as changes in taxonomic diversity, and if the role of the fungal population is known, then functional diversity can also be considered. Although some individual species of fungi are capable of producing many different enzymes, communities that comprise different fungi usually contribute collectively to the decomposition of physically and chemically complex substrates such as leaves (2). Fungal communities vary in species composition from site to site, reflecting fungal versatility and functional resilience and thereby ensuring efficient decomposition and mobilization of nutrients in most environments.

Microfungi are able to degrade virtually all of the organic compounds generated by primary production in the various ecosystems of the world. Moreover, they are also able to degrade xenobiotic compounds, many of which are comparatively new to the environment (3,4). Bacteria also produce a large variety of enzymes in the environment, and an understanding of the interaction between fungi and bacteria is important to comprehension of the decomposition process. In most soils, the fungal biomass corresponds to or exceeds the bacterial component such that fungi play the major role, especially in the initial stages of cellulose, lignin, and chitin decomposition (5). Hyphal growth enables fungi to grow toward and through dense organic material and to grow from one source to another through a depleted zone. Because of the comparatively slow decomposition rate of hyphae, which is due to their high cell wall chitin content, nutrients are immobilized in fungal biomass for a longer period than in bacteria (6). The fungal biomass therefore comprises an impor-

tant soil nutrient pool. Because fungal growth is affected by tillage, fertilization, fungicides, and the removal of plant biomass, fungal biomass in undisturbed, uncultivated soil is normally higher than in cultivated, agricultural soil. As a consequence of the lower fungal biomass, the sustainability (i.e., organic matter content, soil structure, resilience to impacts) of the soil diminishes (6).

II. THE FUNGAL COMMUNITY

The regulation and secretion of fungal extracellular enzymes in pure culture, in vitro, are not reviewed in this chapter; this topic is discussed in detail elsewhere (7,8). Although the fungal enzymes and their principal substrates are well defined, comparatively little is known about their regulation in nature. Some enzymes are induced in the presence of substrates and products, whereas others are regulated by repression/derepression. However, few studies have investigated this recognized but undoubtedly complex situation. One of the reasons for the involvement of the whole fungal community in decomposition could be regulatory factors that do not specifically favor one strain but rather stimulate several fungi to utilize the substrate or components of it. The most important degradative extracellular enzymes produced by fungi are the proteases, amylases, pectinases, cellulases, ligninases, and xylanases, although enzymes such as chitinases, cutinases, phytases, and phosphatases also play a role.

A. Principal Groups of Soil Microfungi

There are two principal taxonomic groups of microfungi active in the decomposition process in litter and soil: the Zygomycetes and the Deuteromycetes. These have various morphological and enzymatic traits that enable them to grow and proliferate on diverse substrates. Examples of functional groups of fungi are shown in Table 1.

Mucorales is the largest group of the Zygomycetes, encompassing such important genera as *Mucor*, *Rhizopus*, *Absidia*, and *Mortierella*. These all have fast-growing mycelia, are devoid of hyphal septa, and exhibit various kinds of vegetative sporangiospores. Some species also produce sexual spores, which are often thick-walled and able to survive under adverse environmental conditions. Members of the Mucorales are unable to degrade polysaccharides such as cellulose and lignin, but they rapidly penetrate organic material and utilize soluble sugars in competition with bacteria during the initial phases of decomposi-

Table 1 Examples of Functional Genera of Fungi in the Key Taxonomic Groups

	Zygomycetes	Ascomycetes	Basidiomycetes	Deuteromycetes
Soil fungi	*Mortierella*	*Peziza*	*Agaricus*	*Trichoderma*
Litter fungi	*Mucor*	*Chaetomium*	*Mycena*	*Cladosporium*
Wood fungi		*Xylaria*	*Trametes*	imperfect stages[a]
Mycorrhizal fungi	*Endogone*	*Tuber*	*Cantharellus*	
Pathogenic fungi	*Enthomophthora*	*Erysiphe*	*Ustilago*	*Verticillium*

[a] Imperfect stages of genera of Ascomycetes and Basidiomycetes.

tion (9). Species of the large genus *Mortierella* have different capacities and are specialized for chitin degradation, producing enzymes such as β-*N*-acetylglucosaminidase (10).

The Deuteromycetes comprise a very large (approximately 17,000 species) and heterogeneous group of filamentous fungi—the hyphomycetes. These only reproduce vegetatively and hence are traditionally referred to as *Fungi Imperfecti*. When a sexual phase is known, the taxon should be classified among the Ascomycetes (or Basidiomycetes), but for identification purposes, the vegetative stages are also included in the Deuteromycetes. The Deuteromycetes are enzymatically extremely versatile and hence often play the major role in fungal degradation of organic matter. Moreover, many species produce a large number of conidia and are fast-growing, thereby spreading rapidly throughout the environment and germinating when conditions are optimal. Many strains are unable to form conidia and remain sterile, and such sterile mycelia may account for up to half of the strains isolated from a site. The Deuteromycetes are known to be capable of producing enzymes important for the decay processes (7). Some pathogenic imperfect fungi are also saprophytic and produce a variety of enzymes, primarily those necessary for penetrating insect cuticles (e.g., chitinase and protease) and cellulase for decomposing plant material. An example of such a versatile fungus is *Paecilomyces farinosus* (11).

Two other fungal groups are also important in the decomposition processes. The first group, the Ascomycetes, includes genera that produce both vegetative conidia and sexual spores (e.g., *Penicillium* and *Aspergillus* spp.) as well as a large group of yeasts common in certain soils and fruits with a high sugar content (9). The second group, the Basidiomycetes, include the wood-decaying fungi with large groups of soft rot, brown rot, and white rot fungi producing lignocellulose-degrading enzymes (12). An important functional group of Basidiomycetes are the ectomycorrhizal fungi, which are in direct mycelial contact with the roots of trees, bushes, and herbs in terrestrial ecosystems (12).

B. Fungal Biomass

Determination of fungal biomass is important for estimating the organic C pool in fungal hyphae; for comparing fungal biomass in different soils and horizons; for determining the effects of pollution and changes in climate and land use; and for using biomass data in decomposition models (13). The mycelium is often well hidden in soil aggregates and is not easily accessible. As a result, many different approaches have been employed to determine fungal biomass, including microscopy, cultivation, substrate utilization, and analyses of structural components. Determination of fungal biomass in litter and soil is usually based on the estimation of fluorescein diacetate (FDA) or cell components such as ergosterol and phospholipid fatty acids (PLFA), procedures that are fully described elsewhere (14,15). A physiological method much used for determining total soil microbial biomass is substrate-induced respiration (SIR) (16), and by combining SIR with antibiotic inhibition the contributions of the bacterial and fungal biomass can be separated (17). Although the selective inhibition technique has the potential to be the most precise means of measuring the active fungal biomass, and many attempts to improve the procedure have been reported, it nevertheless remains very difficult to obtain reliable, reproducible results, especially when using soil samples with a high organic matter content (18–23).

An integrated experiment to demonstrate fungal and bacterial competition was carried out by Hu and van Bruggen (24), who investigated microbial dynamics during the decomposition of cellulose-amended soil. Measuring respiration in combination with the selective inhibition technique, they showed that the fungal population played the major

role in cellulose decomposition since the bacterial respiration was very low during the 30-day experimental period. Fungal respiration peaked within 10 days when the bacteria (and fungi) had depleted the easily available C; after 10 days the fungi initiated cellulose degradation.

A recently developed technique based on the production of the fungal enzyme chitinase, has been employed to estimate fungal presence and activity in soil and litter. Adding a fluorogenic substrate analog, 4-methylumbelliferyl N-acetyl-β-D-glucosamide (MUF), to the sample allows N-acetylglucosaminidase (NAGase) activity to be measured. Laboratory experiments have shown that the NAGase activity is significantly correlated with both the ergosterol content and the fungal PLFA (25,26), thus confirming that fungi are the predominant source of the activity. This method was used to compare the spatial and temporal changes in fungi and fungal enzyme activity during decomposition of maize litter in two agricultural soils from the northern temperate and the southern Mediterranean zones (27). Chitinase activity was determined by the MUF technique on six sampling occasions during one year (25). The level of enzyme activity differed between the two soils; activity was considerably lower and the lag time before production of enzymes longer in the Mediterranean zone soil than in the temperate zone soil. Moreover, enzyme activity was considerably lower in bulk soil than in the ''residuesphere'' (i.e., the interface between soil and plant residues).

Fungal–bacterial interaction during the decomposition of beech leaves was demonstrated by Møller and associates (26), who showed that the chitinase (N-acetylglucosaminidase) activity was fungal in origin and that bacteria made only a marginal contribution to chitin degradation despite the fact that the bacterial community (as revealed by the Biolog method) exhibited high functional diversity. A significant correlation exists between chitinase activity and both exo- and endocellulase activity, possibly indicating that both enzymes are mainly fungal in origin.

The validity of chitinase activity as a measure of fungal biomass was substantiated in a study of the influence of fungal–bacterial interaction on the bacterial conjugation rate in the residuesphere (28). The aim was to determine whether the residuesphere is a hot spot for conjugal gene transfer and whether fungal colonization of the leaves affects conjugation efficiency. In a microcosm experiment with soil and barley straw precolonized by soil fungi, chitinase activity increased after 17 days whereas the number of transconjugants decreased. The activity of chitinase and N-acetylglucosidase as measured by the MUF technique decreased with depth in four different Japanese forest soil profiles (29). It was concluded that the higher levels of these enzymes in the upper part of the profile could be due to the presence of fungi (chitin in the cell walls) and arthropods (chitin in the exoskeleton) serving as substrates.

Enzyme determination using MUF substrates is a highly sensitive technique and the enzymes can be measured in nanomolar concentrations and under in situ conditions. Other MUF substrates have also been used to measure various enzymes in soils and sediments, including cellulases, peptidases, and glucosidases (30–32).

III. INFLUENCE OF RESOURCE QUALITY ON FUNGAL ACTIVITY

The two main plant compounds, cellulose and lignin, are degraded by both bacteria and fungi but the literature on fungal enzymes states that the Basidiomycetes play the major role (33–35). Bacteria are generally unable to degrade lignin completely. Even the Actino-

mycetes, which exhibit mycelial growth, do not have the same lignin-degrading capacity as fungi and do not appear to play a significant part in lignin degradation. Many genera of saprophytic microfungi, which colonize leaf litter during decomposition and operate in the different soil horizons, degrade cellulose and lignin compounds to different degrees. However, this group of microfungi tends to be ignored in many of the reviews of cellulose and lignin degradation.

The lignin content markedly affects the decomposition rate of both leaf and needle litter types; lignin concentration and living fungal biomass are inversely related (36). This indicates that fungal growth during colonization is repressed by lignin and that decomposition rates in humified litter are very low. Entry and Backman (37) also argued that the lignin content of organic matter is a major factor controlling decomposition of organic matter in terrestrial ecosystems. In experiments involving the addition of C and N to forest soils they found that as the C and N concentration increased, so did cellulose and lignin degradation and the active fungal biomass. Fungal biomass correlated with both cellulose and lignin degradation, indicating the importance of the fungal population in the decomposition processes. It was concluded that the cellulose/lignin/N ratio more accurately predicts the rate of organic matter decomposition (and hence substrate quality) than overall C/N ratios.

It is not possible to test microfungi for lignin degradation ability directly. Alternative substrates have been introduced over the years; these include vanilin, indulin, ferrulic acid, and, most importantly, ^{14}C-labeled synthetic lignins. Various fungal enzymes are involved in lignin degradation, including lignin peroxidase, manganese peroxidase, polyphenol oxidases, and especially laccase (34,38–43).

As fungi or other microorganisms capable of attacking humic acid or gallic acid are also able to degrade lignin (44), the effect of these two compounds on microfungi combined with determination of their degradation ability may be used as an indicator of lignin degradation. Gallic acid has been shown to inhibit the growth of fungi isolated from litter and soil from temperate forests. Radial growth of the frequently isolated microfungi (e.g., species of *Cladosporium*, *Aureobasidium*, *Epicoccum*, *Alternaria*, and *Ulocladium*) was restricted on agar containing gallic acid as the sole carbon source as compared with growth on medium devoid of gallic acid. Some *Penicillium* species producing polyphenol oxidase were able to grow in the presence of gallic acid and may be the only fungi able to tolerate gallic acid in the environment (45).

In a study of deciduous forest litter, Rai et al. (46) reported marked inhibition of *Curvularia*, *Cladosporium*, and *Myrothecium* spp. in cultures containing gallic acid. Although most of the isolated strains of these genera are able to produce polyphenol oxidase, only *Penicillium*, *Aspergillus*, and *Trichoderma* spp. were not inhibited and were able to utilize gallic acid as a source of carbon and energy.

In a study of the humic acids–degrading efficiency of fungi and bacteria, Gramass et al. (47) investigated the growth of 36 fungi and 9 bacteria isolated from soil and plant material, including wood-degrading and soil-inhabiting saprophytic Basidiomycetes, ectomycorrhizal fungi, and soil-borne microfungi and bacteria. The wood-degrading Basidiomycetes decomposed humic acid at twice the rate of other groups of fungi, whereas the bacteria exhibited little humic acid degradation.

Decomposition of beech leaves has been investigated by Rihani and associates (48). Pure cultures of two white rot fungal strains (Basidiomycetes), isolated from beech soil and litter, were able to use pectin, cellulose, lignin substitutes, and phenols as their sole carbon source in pure cultures. Thus, when the two strains were inoculated separately on

sterile fresh leaves, cellulose, lignin, and phenol degradation was initiated immediately. Fourteen days later, when 20% of the cellulose had been degraded, the rate of lignin degradation increased. Decomposition was rapid during the first month but virtually ceased after four months.

Low resource quality and adverse environmental conditions (e.g., low water availability) result in low decomposition rates. This has been examined by incubating pine needles in litter bags in a southern Italian pine forest (49). Both the C/N ratio and the lignin content of the litter were high. Measurement of biological parameters such as CO_2 evolution and fungal biomass over a three-year period revealed a significant positive correlation between respiration rate and moisture content of the litter. There was no obvious relationship between fungal biomass and other measured parameters (i.e., litter mass loss, lignin content, and nitrogen content). It was concluded that since the litter was very dry for most of the year, an autochthonous fungal flora had developed that was able to degrade these litter types under adverse conditions albeit at a low rate.

The examples of interactions between substrates and fungal groups mentioned and the influence of different concentrations of substrates illustrate the complex and dynamic processes involved in litter decomposition. In the next section the successional stages of decomposition are discussed in the context of enzyme activity.

IV. FUNGAL POPULATIONS AND ENZYME ACTIVITY

Numerous studies on fungal succession have been published, many of which discuss the identification of fungi at different stages of decomposition (1). However, the emphasis is usually on taxonomic identity rather than on enzymatic diversity. Those genera most frequently mentioned in connection with early colonization of the organic debris in the temperate zone are *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Epicoccum*. In her review, Frankland (1) concluded, ''Let us ecologists not neglect to study in greater depth more of the star performers in fungal succession, on which the maintenance of entire ecosystems may depend.'' In this context ''star performers'' encompass the important enzyme producers and hence the key decomposers.

The link between taxonomic and functional diversity in the fungal population has been discussed in reviews by Miller (50) and Zak and Visser (51), both of which emphasize the importance of succession studies. The relationship between fungal succession and the enzymatic potential of the fungi has been observed during decomposition of forest litter, e.g., of alder (2,52–57) and beech (54–57) (see Table 2).

On beech leaves, fungal species of the genera *Aureobasidium*, *Cladosporium*, *Epicoccum*, and *Alternaria* appear first, although *Mucor*, *Phoma*, and *Acremonium* are often early colonizers (Table 3). *Acremonium* spp. isolates attack cellulose and chitin as well as gallic acid, although the main role of these fungi in the initial phases of decomposition is to degrade pectin and starch. The second wave of degraders varies in different litters, consisting of a wider variety of genera (e.g., *Cylindrocarpon*, *Phialophora*, *Phoma*, and *Phomopsis*). These fungi are versatile with regard to enzyme production and secrete cellulases, polygalacturonases, xylanases, lipases, and proteases. A third group of degraders, which come into play when the litter is almost completely decomposed, chiefly consists of cellulose-degrading fungi but also includes lignin and chitin degraders of genera such as *Trichoderma*, *Penicillium*, *Fusarium*, *Acremonium*, and *Mortierella*. In the later stages

Table 2 The Most Frequent Microfungal Genera in Alder, Ash, and Beech Litter Able to Utilize Pectin, Cellulose, Chitin, and Gallic Acid

| | | | Beech | |
| | | | Year 1 new | Year 2 old |
	Alder	Ash		
Pectin	*Phoma*	*Phomopsis*	*Acremonium*	*Trichoderma*
	Cladosporium	*Phoma*	*Sterile mycelia* dark	*Sterile myelia* hyaline
	Cylindrocarpon	*Epicoccum*	*Aureobasidium*	*Mortierella*
			Heteroconium	*Chrysosporium*
			Cladosporium	*Penicillium*
Cellulose	*Phoma*	*Phoma*	*Acremonium*	*Trichoderma*
	Verticillium	*Cylindrocarpon*	*Heteroconium*	*Acremonium*
	Cylindrocarpon		*Phialophora*	*Mortierella*
Chitin	*Mortierella*	*Phoma*	*Acremonium*	*Mortierella*
	Verticillium			*Trichoderma*
	Aureobasidium			*Penicillium*
Gallic acid	*Cladosporium*	*Phoma*		
	Cylindrocarpon	*Phomopsis*	nd[a]	nd[a]
		Cylindrocarpon		

[a] nd, not determined.
Source: Refs. 2, 53, and 54.

of decomposition, *Mortierella* spp. strains constitute 28% of the isolates, all of which are able to degrade chitin, whereas only a few also attacked pectin and cellulose. *Mortierella* spp. isolates have been tested for the production of hydrolytic enzymes by Terashita and associates (58), who reported that 18 isolates were able to produce acid protease, β-1,3-glucanase and chitinase, whereas cellulase was produced by a smaller number only.

The applicability of laboratory findings to events in the environment depends on how reliably the environmental conditions are simulated in the model and culture studies. Moreover, as isolation procedures for fungi and enzyme assays differ among studies, the findings of different research groups are not always directly comparable. However, the methods used in the cases discussed later concerning forest litter decomposition are almost identical, thereby allowing valid comparisons to be made.

In each of the studies the fungi were isolated by blending soil or litter in water and washing the soil particles to remove conidia. Growing hyphae remained attached to the particles, which were placed on appropriate agar plates and incubated at 10°C or 15°C until growth of the fungal strains was sufficient to allow identification. Although the choice of medium varied, soil fungi, unlike bacteria, are able to grow on both complex and very dilute (oligotrophic) media. Temperature significantly influences enzyme production in the environment, but this influence is difficult to study in situ and most of our knowledge stems from applied studies of enzyme production in the laboratory. Flanagan and Scarborough (44) reported that a fungal strain isolated from an arctic soil and grown on cellulose or pectin as the carbon source produced cellulase at low temperature (4°–5°C), whereas pectinase production was optimal at much higher temperatures (15°–25°C).

Table 3 Microfungal Succession and Substrate Utilization Pattern During Decomposition of Beech Litter over an 18-Month Peroid[a]

Fungal genera	Fresh Leaves	Months				
		3	6	13	16	18
Acremonium spp.	Pe Ce Ch	Pe Ce	Pe	Pe Ce	Pe	
Cladosporium cl.	Pe					
Sterile mycelia black	Pe					
Sterile mycelia black	Pe					
Phialophora sp.	Pe Ce					
Sterile mycelia grey	Pe					
Sterile mycelia dark grey		Pe	Ch			
Aureobasidium pullulans		Pe	Ch			
Heteroconium sp.		Pe Ce				
Cladosporium herbarum		Pe				
Pseudofusarium sp.		Pe				
Sterile mycelia grey		Pe				
Sterile mycelia brown						
Sclerotia			Ce			
Trichoderma viride				Pe Ce Ch		Ce Ch
Mortierella spp.				Pe Ce Ch	Ch	Pe Ce Ch
Penicillium spp.					Ce	Pe Ce Ch
Sterile mycelia hyaline					Pe	
Chrysosporium sp.					Pe	
Mortierella vinaceae					Pe	

a Pe, pectin; Ce, Cellulose; Ch, chitin.
Source: Ref. 54.

A. Forest Litter Decomposition

The literature on decomposition encompasses numerous studies on many different types of forest litter from all over the world. Both recent and older reports concentrate on temperate forest litter (mostly from deciduous forests); tropical litter is only rarely included. Research on fungal activity and carbon sequestration in relation to the high decomposition rate in tropical rain forest should thus be accorded high priority in future studies.

Alder litter was investigated by Rosenbrock et al. (52), who showed that fungal amylase, polygalacturonidase, cellulase, xylanase, pectinase, protease, and laccase were produced at the beginning of the decomposition period. A high proportion of the fungal isolates produced amylase (80–100%) and polygalacturonase (50–95%) throughout the first year of decomposition, whereas the percentages of fungi producing cellulase, xylanase, pectinlyase, protease, and lipase decreased with time. Pectinase and protease were only produced by approximately half of the isolated strains. Laccase activity was restricted to only 2–6% of the isolates and occurred sporadically throughout the year. After the initial dominance of *Mucor*, *Alternaria*, and *Epicoccum* spp. these fungi were displaced by a number of different *Fusarium* species.

The potential of fungi to produce a large range of various enzymes during the initial stages of litter decay was also observed in our own decomposition studies of alder, ash, and beech litter. In the beech litter study (54), fungal strains were isolated and tested on pectin, cellulose, and chitin. Three months after litter fall, 90% of the isolates were recorded as pectinase producers, e.g., *Aureobasidium* and *Cladosporium* spp; *Heteroconium* and *Acremonium* spp. were able to degrade both pectin and cellulose. After 10 months the proportion of pectinase-positive fungi had decreased to 40%, whereas the proportion of cellulose-decomposing fungi had increased from 20% to 60%, the latter mainly accounted for by various sterile mycelia. After 18 months the active fungal flora was dominated by *Mortierella*, *Penicillium*, and *Trichoderma*, which degrade both cellulose and chitin. At this stage a single fungal strain could be highly versatile, able to attack more than one polymer (and its lower-molecular-mass oligomers). This study thus demonstrates the occurrence of taxonomic and functional succession during decomposition of beech litter, as the fungal flora change composition and functional role as the substrate resource is depleted.

Fungal succession and decay of beech litter were investigated in a transect/transplant experiment in four European beech forests in the (CANIF) project (57,59). Fungal activity was measured as endo- and exocellulase activity (endo 1,4-β-glucanase/exo cellobiohydrolase) using a MUF substrate, 4-methylumbelliferyl β-D-lactoside (27). Although the MUF technique does not distinguish between fungal and bacterial cellulase activity, Møller and coworkers (26) showed that the cellulase activity measured by the MUF technique is mainly fungal in origin with very few bacteria active. Moreover, Miller et al. (25) showed that MUF cellulase activity correlated with ergosterol and fungal PLFA, Cotrufo and colleagues (59) reported a correlation of cellulase activity with decomposition rate (litter weight loss). Thus the MUF cellulase activity measured probably reflects the activity of live fungi colonizing the litter. In the CANIF project, fungal strains were isolated and identified and the Simpson diversity index was calculated (60). In the transect experiment, samples of leaves from an Italian beech forest were placed in the litter layer of beech forests in France, Germany, and Denmark. In the transplant experiment, beech leaves from these three forests were placed together with the local litter in the Italian beech forest. By incubating identical litter types in different climatic zones and by placing litter of

different origin in the same climate, interesting decay patterns and biodiversity changes were revealed. A linear regression model of mass loss as a function of cumulative cellulase activity for pooled data from all sites (both transplant and transect) revealed a significant correlation between the two sets of data (59).

When the three types of ''foreign'' litter were placed at the southern site in Italy, both cellulase activity and fungal diversity were lower than in the native litter layer. The Italian litter had the highest cellulase activity but the lowest fungal diversity, thus indicating that the fungal population was adapted to the local climate and soil. Another interesting finding was that when the Italian litter was placed along the transect in France, Germany, and Denmark, the rate of cellulase activity increased to much higher levels than when incubated in Italy, especially during the second year of decomposition. When the litter was placed in a less adverse climate, decomposition proceeded at a higher rate. At the Danish site, for example, decomposition was twice as fast as in Italy. Fungal diversity was high during the first months but diminished with time, while the cellulase activity remained high. Key functions are undertaken by different fungi at different sites and stages of decomposition, thus indicating the occurrence of functional substitution. The most frequent fungi on the Italian beech litter were *Chalara* species, which initially constituted 40% of the population but disappeared rapidly after the first eight months of decomposition. *Cladosporium* and *Aureobasidium* spp. were present during the entire period, whereas *Chalara* sp. was replaced by cellulase-producing fungi such as *Penicillium*, *Acremonium*, and *Alternaria* spp., and at the Danish site also by *Trichoderma* sp. At no time was it possible to correlate fungal diversity with decomposition rate as measured by cellulase activity.

In two significant papers, Andrén et al. (61,62) discussed biodiversity and species redundancy among litter decomposers and the influence of soil microorganisms on ecosystem-level processes. Some of the hypotheses put forward in Andrén (61) are relevant to the CANIF data, for example, the hypothesis ''If diversity is important, there should be a positive correlation between diversity and decomposition rate. . . .'' When closely examining the preceding findings it is apparent that fungal diversity was low in all four types of litter when ''foreign'' beech litter was incubated in Italy. On each sampling occasion during the two-year study period the transplant experiment also revealed low cellulase activity. In the transect experiment, however, in which Italian litter was placed in France, Germany, and Denmark, a different picture emerged. Thus the cellulase activity increased at all sites during the incubation period, and the highest level of activity was reached during the second year of decomposition. Simultaneously, fungal diversity was initially high but decreased to very low levels toward the end of the decomposition period, lower than in the transplant experiment. As a consequence, fungal diversity and decomposition activity were inversely correlated. The difference in the results of the two experiments may be attributable to a number of factors. For example, decomposition of cellulose seems to proceed well under conditions of low diversity.

Another hypothesis put forward by Andrén (61) was, ''If a particular organism group controls decomposition, it should be possible to relate its dynamics to decomposition rate. . . .'' This was demonstrated for the fungal community in the preceding experiments. If the fungal populations are removed from the litter or their growth is inhibited from the beginning of the decomposition process, however, the decomposition proceeds extremely slowly and is solely due to bacterial cellulase activity (26).

A third hypothesis proposed by Andrén (61) can be summarized as follows: ''During decomposition there is a succession of fungi adapted to changes in substrate quality but the decomposition rate may nevertheless remain constant.'' The question here is whether

a change in the succession observed in the two experiments mentioned will affect the litter decay rate. There is a marked succession of fungi, but would a change in the composition of the fungal flora at a certain stage affect the decomposition rate? This is difficult to answer since it is not easy to manipulate natural systems and exclude specific members of the fungal succession.

The paradox of the apparent simplicity of ecosystem process control and the high diversity of soil organisms (invertebrates, bacteria, and microfungi) has been discussed (62). Although the most simple decomposition models operate without including diversity, the microbial component, expressed, for example, as microbial biomass, may still be able to predict the turnover of organic matter. Most decomposition models include components such as temperature, moisture, and resource quality, and since these variables have direct effects on microbial growth and activity, they are also important for decomposition. When a more detailed view is necessary, enzyme production seems to be a useful tool. Fungal enzyme production is essential to decomposition, and it is thus important to study the response of individual fungi to environmental changes.

B. Decomposition of Crop Residues

Postharvest decomposition of crop litter plays an import role in returning nutrients to agricultural soils. Much research has been undertaken to determine the effect of land use changes, management practices, and resource quality on litter decomposition. Some examples of effects on the fungal community and enzyme production are examined later in order to highlight the role played by microfungi in the sustainability of agricultural soils. As previously discussed, the fungal community of agricultural soil is under stress due to the management procedures employed in modern agriculture, and fungal biomass is consequently much lower than in natural soils (6). It was stated that agricultural practice especially would affect the fungal biomass. Since fungal hyphae and fungal production of polysaccharides are essential for soil stability, consequences could include less stable soil aggregates.

The effect of reduced soil management on fungal activity in agricultural soil was investigated in laboratory experiments in which maize litter was either placed on top of or incorporated into the soil to simulate reduced and a traditional soil management practice, respectively (27). Cellulase activity and chitinase activity in the bulk soil were both low. When maize litter was incorporated into the soil, enzyme activities increased. When the litter was placed on top of the soil, the level of activity was consistently higher. Moreover, fungal isolation frequency was also higher (i.e., more fungi per soil particle) and the fungal communities were more diverse. However, after one year, the total degree of mineralization of maize litter was the same irrespective of whether the litter had been placed on top of the soil or incorporated into it. The fungal community on maize litter was initially dominated by members of the Mucorales, e.g., species of *Mucor*, *Mortierella*, and including *Rhizopus*, genera that are only occasionally found in soil. After three months the Mucorales were replaced by cellulolytic fungi such as *Fusarium*, *Acremonium*, and *Penicillium* (27).

During decomposition of maize litter in soil, protease, xylanase, and invertase activities were two orders of magnitude higher than in control soil (63). These enzymes are not specific for fungi, however, and the authors made no attempt to distinguish between fungal and bacterial activities. Some crop litter components decompose very slowly. The decomposition rates of different components of wheat litter (internodes and leaves) in the

soil have been compared in an eight-month incubation study by Robinson and colleagues (64). A high lignin content (10%) of the initial dry matter and a high C/N ratio (approximately 100) resulted in a low decomposition rate. At the end of the experiment the lignin and cellulose contents were still high in the internodes, whereas the leaves had decomposed almost completely. The fungal genera acting on the two components were cellulose-decomposing fungi such as *Trichoderma* and *Cladorrhinum* spp. In the internodes these two were supplemented with other cellulose decomposers such as *Fusarium*, *Phoma*, and *Penicillium* spp. On the leaves, in contrast, they were supplemented by genera capable of degrading both cellulose and lignin, e.g., *Epicoccum* and *Cladosporium* spp. Ligninolytic Basidiomycetes can be expected to appear much later to complete the decomposition of the internodes.

In similar experiments, Bowen and Harper (65,66) examined the succession of saprophytic microfungi on decomposing wheat straw in agricultural soil during a one-year period together with the cellulase- and lignin-degrading ability of the fungi. The first colonizers were *Mucor* and *Cladosporium* spp. The number of *Mucor* spp. isolates decreased after the first months of decomposition, whereas *Cladosporium* spp. remained abundant. Other fungi played an important role after the first month, e.g., *Penicillium* and particularly *Fusarium* spp. *Chaetomium* was abundant during the first months but was subsequently functionally replaced by *Trichoderma* spp. The fungi that became more abundant as decomposition progressed were tested for their ability to degrade cellulose, lignin, and phenolic acids; it turned out that they were only able to degrade cellulose. Later-appearing Basidiomycetes, which included a *Typhola* sp., were able to degrade both cellulose and lignin, although the lignin decomposition rate differed among the individual fungi. It was argued that these Basidiomycetes with multiple degradation abilities degrade the straw more efficiently than the strong cellulolytic, nonlignolytic, filamentous fungi and hence may play an important role in agricultural soil sustainability. It was also demonstrated that mixed communities of cellulose- and lignin-degrading fungi almost always exhibited higher rates of decomposition than single strains of efficient degraders.

The effect of nitrogen availability on enzyme activity during decomposition of wheat straw in soil has been examined in a two-month study by Henriksen and Breland (67). The carbon mineralization rate was reduced in straw-amended soil having a low N content (less than 1.2% of straw dry weight). Moreover, when the fungal biomass decreased, exocellulase, endocellulase, and hemicellulase activity also decreased. These findings demonstrate the need for available N to improve enzyme production and decomposition of recalcitrant substrates.

The two methodological approaches used, i.e., the testing of strains and the extraction of enzymes, provide complementary information on enzyme production by emphasizing the potential of the living hyphae and the sum of past and present activities respectively. The use of MUF-linked substrates allows work to be undertaken with small samples.

V.   ENVIRONMENTAL IMPACT ON ENZYME ACTIVITY

Many changes in the physical and chemical environment influence fungal activity in the soil. The following examples from the recent literature highlight specific cases. Both environmental and anthropogenic stresses are important. Other chapters of the present volume

(see Chapters 17–20) examine pertinent aspects of this such as pesticides, xenobiotics, heavy metals, and various other pollutants.

Environmental conditions and specific stress factors can markedly affect microbial enzyme activity in the soil. For example, freezing and thawing enhance bacterial and fungal phosphatase, urease, xylanase, and cellulase activity, thereby accelerating decomposition compared with that during continuous snow cover (68).

Cellulase activity is a key element of decomposition, and the determination of this predominantly fungal enzyme is essential in both general decomposition studies and in studies of the effect of stress factors. The impact of industrial pollution on cellulase activity has been investigated in forest humus in northern Finland by two methods (69). The use of cellulose strips inserted into the soil proved to be much less efficient at detecting differences in cellulase activity than traditional, chemical analyses in the laboratory. Only the latter analyses were sufficiently sensitive to demonstrate pollution-induced changes. Cellulase activity correlated well with basal respiration, decreasing significantly with increasing pollution.

An effect of pollution on enzyme activity has also been observed in a study of cellulase, amylase, and invertase activity in litter from coniferous and deciduous trees near a busy highway in northeast India (70). The enzyme activities were much higher in the litter at a site 500 m away from the highway than at a site immediately beside the highway, where decomposition of the polluted litter close to the highway decrease. Cellulase and amylase activities were significantly correlated with the number of fungi and bacteria present.

The antibiotic tylosin has been shown to stimulate soil fungal biomass in soil as measured as chitinase activity by the MUF technique. Thus chitinase activity was higher after 10 days of exposure, and although the level decreased somewhat after 20 days, it remained higher than in untreated soil (71). Mercury at various concentrations had no significant effect on chitinase activity; only a minor decrease was observed at high mercury concentrations (511 µg Hg g^{-1} dry soil) (72).

The activity of a number of soil enzymes has been studied in a grassland soil contaminated with various heavy metals, including As, Cd, Cr, Cu, Ni, Pb, and Zn (73). Total microbial biomass, fungal biomass, and bacterial biomass were also determined. Both biomass and enzyme activity were inversely proportional to the level of contamination, and there was a high degree of correlation between enzyme activities and both SIR and fungal length. The respiration rate and cellulolytic activity of some cellulose-decomposing fungi isolated from salinized Egyptian soils were found to decrease at increasing salinity (74). Although fungi are normally considered to be tolerant of high salt concentrations, this study indicates that the decomposition rate of organic matter is lower in salinized soil. Salinization is a global phenomenon of increasing extent as a result of the drier climate in some areas and especially the impact of human activities.

The effect of the increasing atmospheric concentration of CO_2 has also been focused on in recent years. In a study of the effect of three-year exposure to elevated CO_2 levels on the activity of some of the enzymes essential to the decomposition process, Moorhead and Linkins (75) found that cellulase activity increased in ectomycorrhizae in an arctic tussock soil but decreased in the surrounding soil. They concluded that the decrease in cellulase activity would reduce cellulose turnover by 45%, leading to ''a substantial increase in activities associated with nutrient acquisition by plants and microorganisms, a reduction in litter cellulose decay and an increase in soil mineral nutrient size.'' Dhillion and associates (76) reported an increase in saprophytic fungal hyphal length and cellulase

activity in a Mediterranean soil under conditions of elevated CO_2, thus suggesting stimulation of organic matter decomposition.

The impact of enhanced ultraviolet-B (UV-B) radiation on the decomposition of plant material has been investigated by Gehrke et al. (77), who reported that the lignin content of the plant material decreased with time but at a lower rate than in nonirradiated material. The total microbial respiration rate decreased after exposure to UV-B radiation, but only transiently. Changes in the fungal community indicate that some genera were sensitive to the radiation (*Mucor hiemale* and *Truncatella truncata*), and others were indifferent (*Penicillium brevicompactum*). Although UV-B radiation may influence fungal communities, insufficient information is available to allow any conclusion to be drawn concerning the implications for decomposition.

VI.   CONCLUSIONS

Decomposition was extensively examined by Swift et al. (5). Selected parts of their text focusing on litter quality, the influence of environmental factors, and mathematical modeling were updated by the same authors (78). Although decomposer organisms are not specifically examined by the authors, their conclusions pose three important questions: First, to what extent do decomposer organisms adapt to reductions in diversity by increasing their functional niche? Second, do general relationships exist between species diversity and decomposer function? Third, are there definable levels of diversity at which decomposer processes change significantly? Some of these questions are addressed in the recent literature.

Numerous recent environmental studies have provided evidence concerning fungal enzymes and their regulation, promoting a much more complete understanding of the role of fungal consortia and communities in the decomposition process. The various functional groups of fungi are active in ecosystem niches. The hyphomycetes in particular produce important enzymes able to degrade the plant polymers. Mycologists have traditionally analyzed these fungi on the basis of their morphological characteristics and appearance in a decomposer sequence. Now new methods allow a more detailed analysis of enzyme production and activity. The capacity of many fungi to perform the same hydrolyses on a particular substrate appears to be a less efficient way of organizing the decomposition process. Given the important role of decomposition in nutrient cycling, this overlap in functionality should be viewed as a necessity to ensure decomposition under all circumstances.

In a global change context, it is of great concern whether the dynamic balance among the different functions or enzymes that constitute the decomposition process will continue under changed climatic and soil conditions and changes in land use. It could be hypothesized that a warmer climate will not be accompanied by an increase in cellulose decomposition as great as the increase in lignin decomposition and that the composition and quality of soil organic matter would consequently change. The decomposition of less common compounds could become more susceptible to changes with loss or addition of certain fungal activities. Analysis of all the elements of the decomposition process is therefore important for an overall assessment of the effects of global change.

Considerably more is known about bacterial enzymes than about fungal enzymes, and although the functional diversity of bacteria is greater than that of fungi, fungi are nevertheless most important for the decomposition of plant material. When a greater number of molecular methods have been more widely applied for studying fungal gene activity

and in situ determination of fungal enzyme synthesis, it will be possible to clarify the interactions among enzymes and substrates, and among fungi and other organisms. This approach is already used in plant pathology and will undoubtedly form the basis for many new decomposition studies. Most previous research on molecular microbial ecology has been carried out with soil and rhizosphere bacteria, often with a view to protecting plants against fungal attack. Extending this type of study to litter fungi would provide useful information on the regulation of enzyme synthesis and interaction with other organisms. Besides enhancing our knowledge of ecosystem function, it would also pave the way for new applications in agriculture and industry.

REFERENCES

1. JC Frankland. Fungal succession—unravelling the unpredictable. Mycol Res 102:1–15, 1998.
2. A Kjøller, S Struwe. Microfungi of decomposing red alder leaves and their substrate utilization. Soil Biol Biochem 12:425–431, 1980.
3. UD Singh, N Sethunathan, K Raghu. Fungal degradation of pesticides. Handbook Appl Mycol Soil Plants 1:541–558, 1991.
4. S Kremer, H Anke. Fungi in bioremediation. In: T Anke, ed. Fungal Biotechnology. Weinheim: Chapman & Kall, 1997, pp 276–290.
5. MJ Swift, OW Heal, JM Anderson. Decomposition in terrestrial ecosystems. Oxford: Blackwell, 1979, pp 1–372.
6. PD Stahl, TB Parkin, M Christensen. Fungal presence in paired cultivated and uncultivated soils in central Iowa, USA. Biol Fertil Soils 29:92–97, 1999.
7. WM Fogarty, CT Kelly. Microbial enzymes and biotechnology. 2nd ed. London: Elsevier Applied Science, 1990, pp 1–472.
8. DB Finkelstein, C Ball. Biotechnology of Filamentous Fungi: Technology and Products. Boston: Butterworth-Heinemann, 1992, pp 1–520.
9. MJ Carlile, SC Watkinson. The Fungi. London: Academic Press, 1996, pp 1–482.
10. A Hodge, IJ Alexander, GW Gooday. Chitinolytic enzymes of pathogenic and ectomycorrhizal fungi. Mycol Res 99:935–941, 1995.
11. S Harney, P Widden. Physiological properties of the entomopathogenic hyphomycete Paecilomyces farinosus in relation to its role in the forest ecosystem. Can J Bot 69:1–5, 1991.
12. JW Deacon. Modern Mycology. 3rd. ed. Oxford: Blackwell, 1997, pp 1–303.
13. DS Powlson. The soil microbial biomass: Before, beyond and back. In: K Ritz, J Dighton, KE Giller, eds. Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities. Chicester: John Wiley & Sons, 1994, pp 3–20.
14. JC Frankland, J Dighton, L Boddy. Methods for studying fungi in soil and forest litter. In: R Grigorova, JR Norris, eds. Methods in Microbiology. London: Academic Press, 1990, pp 343–403.
15. A Tunlid, DC White. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. Soil Biochem 7:229–262, 1992.
16. JPE Anderson, KH Domsch. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221, 1978.
17. JPE Anderson, KH Domsch. Measuring of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Can J Microbiol 21:314–322, 1975.
18. K Sakamoto, Y Oba. Effect of fungal to bacterial biomass ratio on the relationship between CO_2 evolution and total soil microbial biomass. Biol Fertil Soils 17:39–44, 1994.
19. J Alphei, M Bonkowski, S Scheu. Application of the selective inhibition method to determine bacterial:fungal ratios in three beechwood soils rich in carbon—Optimization of inhibitor concentrations. Biol Fertil Soils 19:173–176, 1995.

20. CK Johnson, MF Vigil, KG Doxtader, WE Beard. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biol Biochem 28:427–432, 1996.
21. H Velvis. Evaluation of the selective respiratory inhibition method for measuring the ratio of fungal: bacterial activity in acid agricultural soils. Biol Fertil Soils 25:342–360, 1997.
22. HH Schomberg, JL Steiner. Estimating crop residue decomposition coefficients using substrate-induced respiration. Soil Biol Biochem 29:1089–1097, 1997.
23. Q Zhang, JC Zak. Potential physiological activities of fungi and bacteria in relation to plant litter decomposition along a gap size gradient in a natural subtropical forest. Microb Ecol 35: 172–179, 1998.
24. S Hu, AHC van Bruggen. Microbial dynamics associated with multiphasic decomposition of ^{14}C-labeled cellulose in soil. Microb Ecol 33:134–143, 1997.
25. M Miller, A Palojärvi, A Rangger, M Reeslev, A Kjøller. The use of fluorogenic substrates to measure fungal presence and activity in soil. Appl Environ Microbiol 64:613–617, 1998.
26. J Møller, M Miller, A Kjøller. Fungal-bacterial interaction on beech leaves: Influence on decomposition and dissolved organic carbon quality. Soil Biol Biochem 31:367–374, 1999.
27. M Miller. An enzymatic approach in soil microbial ecology, with special emphasis on fungal presence and activity. PhD dissertation, University of Copenhagen, 1998.
28. G Sengeløv. Influence of fungal-bacterial interactions on bacterial conjugation in the residuesphere. FEMS Microbiol Ecol 31:39–45, 2000.
29. H Ueno, K Miyashita, Y Sawada, Y Oba. Assay of chitinase and N-acetylglucosaminidase activity in forest soils with 4-methylumbelliferyl derivatives. Z Pflanzenernähr Bodenk 154: 171–175, 1991.
30. HTS Boschker, TE Cappenberg. A sensitive method for using 4- methylumbelliferyl-β-cellobiose as a substrate to measure (1,4)-β-glucanase activity in sediments. Appl Environ Microbiol 60:3592–3596, 1994.
31. C Freeman, G Liska, NJ Ostle, SE Jones, MA Lock. The use of fluorogenic substrates for measuring enzyme activity in peatlands. Plant Soil 175:147–152, 1995.
32. C Freeman, GB Nevison, S Hughes, B Reynolds, J Hudson. Enzymic involvement in the biogeochemical responses of a Welsh peatland to a rainfall enhancement manipulation. Biol Fertil Soils 27:173–178, 1998.
33. P Markham, MJ Bazin. Decomposition of cellulose by fungi. In: DK Arona, B Rai, KG Mukerji, GR Knudsen, eds. Handbook of Applied Mycology: Soil and Plants. Vol. 1. New York: Marcel Dekker, 1991, pp 379–424.
34. JA Buswell. Fungal degradation of lignin. In: DK Arona, B Rai, KG Mukerji, GR Knudsen, eds. Handbook of Applied Mycology: Soil and Plants. Vol. 1. New York: Marcel Dekker, 1991, pp 425–480.
35. KE Hammel. Fungal degradation of lignin. In: G Cadish, KE Giller, eds. Driven by nature: Plant litter quality and decomposition. Oxon: CAB International, 1997, pp 33–46.
36. B Berg. FDA-active fungal mycelium and lignin concentrations in some needle and leaf litter types. Scand J For Res 6:451–462, 1991.
37. JA Entry, CB Backman. Influence of carbon and nitrogen on cellulose and lignin degradation in forest soils. Can J For Res 25:1231–1236, 1995.
38. S Criquet, S Tagger, G Vogt, G Iacazio, J Le Petit. Laccase activity of forest litter. Soil Biol Biochem 31:1239–1244, 1999.
39. C Chasseur. Étude de la dynamique fongique dans le processus de décomposition de la litière de Fagus sylvatica, pour 2 forets de Bassin de Mons (Belgique) partage du substrat au sein des groupes successionnels. Belg Bot 125:16–28, 1992.
40. A Leonowicz, J-M Bollag. Laccases in soil and the feasibility of their extraction. Soil Biol Biochem 19:237–242, 1987.
41. ML Niku-Paavola, L Raaska, M Itävaara. Detection of white-rot fungi by a nontoxic stain. Mycol Res 94:27–31, 1990.

42. PJA Howard, CH Robinson. The use of correspondence analysis in studies of successions of soil organisms. Pedobiologia 39:518–527, 1995.
43. I Nioh, T Isobe, M Osada. Microbial biomass and some biochemical characteristics of a strongly acid tea field soil. Soil Sci Plant Nutr 39:617–626, 1993.
44. PW Flanagan, A Scarborough. Physiological groups of decomposer fungi on tundra plant remains. In: AJ Holding, QW Heal, SF Maclean Jr, PW Flanagan eds. Soil Organisms and Decomposition in Tundra. Fairbanks: University of Alaska, 1974, pp 159–181.
45. NJ Dix. Inhibition of fungi by gallic acid in relation to growth on leaves and litter. Trans Br Mycol Soc 73:329–336, 1979.
46. B Rai, RS Upadhyay, AK Srivastava. Utilization of cellulose and gallic acid by litter inhabiting fungi and its possible implication in litter decomposition of tropical deciduous forest. Pedobiologia 32:157–165, 1988.
47. G. Gramass, D Ziegenhagen, S. Sorge. Degradation of soil humic extract by wood- and soil-associated fungi, bacteria, and commercial enzymes. Microb Ecol 37:140–151, 1999.
48. M Rihani, E Kiffer, B Botton. Decomposition of beech leaf litter by microflora and mesofauna. I. In vitro action of white-rot fungi on beech leaves and foliar components. Eur J Soil Biol 31:57–66, 1995.
49. A Fioretto, A Musacchio, G Andolfi, V De Santo. Decomposition dynamics of litters of various pine species in a Corsican pine forest. Soil Biol Biochem 30:721–727, 1998.
50. S Miller. Functional diversity in fungi. Can J Bot 73(Suppl):50–57, 1995.
51. JC Zak, S Visser. An appraisal of soil fungal biodiversity: The crossroads between taxonomic and functional biodiversity. Biodiversity Conservations 5:169–183, 1996.
52. P Rosenbrock, F Buscot, JC Munch. Fungal succession and changes in the fungal degradation potential during the initial stage of litter decomposition in a black alder forest (Alnus glutinosa (L.) Gaertn.). Eur J Soil Biol 31:1–11, 1995.
53. A Kjøller, S Struwe. Functional groups of microfungi on decomposing ash litter. Pedobiologia 30:151–159, 1987.
54. A Kjøller, S Struwe. Decomposition of beech litter: A comparison of fungi isolated on nutrient rich and nutrient poor media. Trans Mycol Soc Japan 31:5–16, 1990.
55. A Kjøller, S Struwe. Functional groups of microfungi in decomposition. In: GC Carroll, DT Wicklow, eds. The Fungal Community. New York: Marcel Dekker, 1992, pp. 619–630.
56. A Kjøller, S Struwe. Analysis of fungal communities on decomposing beech litter. In: RK Ritz, J Dighton, KE Giller, eds. Beyond the biomass: Compositional and functional analysis of soil microbial communities. Chicester: John Wiley & Sons, 1994, pp. 191–199.
57. A Kjøller, M Miller, S Struwe, V Wolters, A Pflug. Diversity and role of microorganisms. In: E Schulze, ed. Carbon and Nitrogen Cycling in European Forest Ecosystems: Ecological Studies. Vol. 142. Heidelberg: Springer Verlag, 2000, pp. 382–402.
58. T Terashita, T Sakai, K Yoshikawa, J Shishiyama. Hydrolytic enzymes produced by the genus Mortierella. Trans Mycol Soc Jpn 34:487–494, 1993.
59. MF Cotrufo, M Miller, B Zeller. Litter decomposition In: E Schulze, ed. Carbon and Nitrogen Cycling in European Forest Ecosystems: Ecological Studies. Vol. 142. Heidelberg: Springer Verlag, 2000, pp 276–296.
60. AE Magurran. Ecological Diversity and Its Measurement. Princeton, NJ: Princeton University Press, 1988, pp 1–179.
61. O Andrén, J Bengtsson, M Clarholm. Biodiversity and species redundancy among litter decomposers. In: HP Collins, GP Robertson, MJ Klug, eds. The Significance and Regulation of Soil Biodiversity. Netherlands: Klüwer Academic, 1995, pp 141–151.
62. O Andrén, L Brussard, M Clarholm. Soil organism influence on ecosystem-level processes—bypassing the ecological hierarchy? Applied Soil Ecology 11:177–188, 1999.
63. E Kandeler, J Luxhøi, DV Tscherko, J Magid. Xylanase, invertase and protease at the soil-litter interface of a loamy sand. Soil Biol Biochem 31:1171–1179, 1999.

64.   CH Robinson, J Dighton, JC Frankland, JD Roberts. Fungal communities on decaying wheat straw of different resource qualities. Soil Biol Biochem 26:1053–1058, 1994.
65.   RM Bowen, SHT Harper. Fungal populations on wheat straw decomposing in arable soils. Mycol Res 93:47–54, 1989.
66.   RM Bowen, SHT Harper. Decomposition of wheat straw and related compounds by fungi isolated from straw in arable soils. Soil Biol Biochem 22:393–399, 1990.
67.   TM Henriksen, TA Breland. Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biol Biochem 31:1121–1134, 1999.
68.   F Schinner. Litter decomposition, CO_2-release and enzyme activities in a snow bed and on a windswept ridge in an alpine environment. Oecologia (Berlin) 59:288–291, 1983.
69.   R Ohtonen, P Lähdesmäki, AM Markkola. Cellulase activity in forest humus along an industrial pollution gradient in Oulu, Northern Finland. Soil Biol Biochem 26:97–101, 1994.
70.   SR Joshi, GD Sharma, RR Mishra. Microbial enzyme activities related to litter decomposition near a highway in a sub-tropical forest of North East India. Soil Biol Biochem 25:1763–1770, 1993.
71.   AK Müller, K Westergaard, S Christensen, SJ Sørensen. The effect of long-term mercury pollution on the soil microbial community. FEMS Microbiol Ecol 36:11–19, 2001.
72.   AK Müller, K Westergaard, S Christensen, SJ Sørensen. The diversity, structure and function of soil microbial communities exposed to different disturbances. OIKOS 2001.
73.   RG Kuperman, MM Carreiro. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biol Biochem 29:179–190, 1997.
74.   RAM Badran. Cellulolytic activity of some cellulose-decomposing fungi in salinized soils. Acta Mycol 29:245–251, 1994.
75.   DL Moorhead, AE Linkins. Elevated CO_2 alters belowground exoenzyme activities in tussock tundra. Plant Soil 189:321–329, 1997.
76.   SS Dhillion, J Roy, M Abrams. Assessing the impact of elevated CO_2 on soil microbial activity in a Mediterranean model ecosystem. Plant Soil 187:333–342, 1996.
77.   C Gehrke, U Johanson, TV Callaghan, D Chadwick, CH Robinson. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in Vaccinium leaves from the Subarctic. Oikos 72:213–222, 1995.
78.   OW Heal, JM Anderson, MJ Swift. Plant litter quality and decomposition: A historical overview. In: G Cadish, KE Giller, eds. Driven by Nature: Plant Litter Quality and Decomposition. Oxon: CAB International, 1997, pp 3–30.